

THE GENETICS OF CURLY WING IN DROSOPHILA,
ANOTHER CASE OF BALANCED LETHAL
FACTORS

LENORE WARD

4404 Morningside Avenue, Sioux City, Iowa

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LENORE WARD

4404 Morningside Avenue, Sioux City, Iowa

A dissertation submitted in partial fulfillment
of the requirements for the degree of Doctor of
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LENORE WARD

4404 Morningside Avenue, Sioux City, Iowa

Received January 3, 1923

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INTRODUCTION

Curly wing, with which this paper deals, is the first easily and sharply separable dominant wing character that has been found in the second linkage group of *Drosophila melanogaster*. It maintains itself in a balanced lethal stock that has arisen during selection. In out-crosses this stock produces twin hybrids which offer a parallel to cases in *Oenothera*; and the inbred stock occasionally yields, on account of crossing over, apparent vestigial and cinnabar² mutants. Analysis of this stock shows that one second-chromosome homologue carries the gene for the dominant character curly. In homozygous condition the curly gene acts as a lethal, except in rare cases in individuals which survive as dwarfed but fertile flies that are very curly.

¹ Contribution from the Zoölogical Laboratory of the UNIVERSITY OF MICHIGAN.

The homologue that carries curly carries also a new recessive eye color, namely, cinnabar², which is a less extreme allelomorph of the original cinnabar. The other homologue carries a recessive character, vestigial, and, about ten units to the left of vestigial, a recessive lethal. The vestigial flies are nearly all sterile, which may mean that the vestigial is a new nearly sterile allelomorph of vestigial (viz., v_{θ}^2), or that there is a sterility factor also in the homologue with vestigial.

In each of the two second chromosomes, there are dominant factors which inhibit crossing over along the entire length of the second chromosome under normal temperature conditions. Very rarely a case of crossing over does occur at normal temperatures; but at high temperatures ($30^{\circ} \pm$) crossing over in the right half of chromosome II becomes fairly frequent (6 percent $\pm$). Double and triple crossovers form a disproportionately large percentage of the total of such crossovers. Curly, or more probably the crossover suppressors that are in the chromosome with curly, appear to reduce the amount of crossing over in chromosomes I and III. It is probable that the crossover modifiers in the vestigial homologue are identical with those in the curly homologue, and that curly arose in a stock homozygous for these modifiers.

The excellent viability of curly, its ease of identification, dominance, and property of preventing crossing over in the second chromosome at normal temperatures make it a useful character in experiments and in the "balancing" of other stocks that would otherwise require selection each generation.

ACKNOWLEDGMENTS

This investigation was carried on under the direction of Professor A. F. SHULL, and I am indebted to him for much assistance. Dr. H. J. MULLER has rendered valuable aid throughout the work, and Professor ROY E. CLAUSEN has made many valuable suggestions. The paper has also been examined by Dr. C. B. BRIDGES, who has checked up the linkage data, and has suggested new interpretations for some of the findings.

SOURCE OF CURLY

The original wild stock from which curly came was collected in Ann Arbor, Michigan, in September, 1918, and kept for use as wild stock. It eventually produced three entirely wingless females. These females were crossed to wild and the F_1 were all normal flies. The inbred F_1 produced an F_2 chiefly wild, but a few with crumpled wings. These abnormal-winged flies were mated together, and again gave wild and abnormal flies.

This practice of selecting the abnormal-winged type of fly each generation, for matings, was continued, with increasing proportion of abnormal wings of several different types, until abnormal types constituted about 50 percent of the total number of flies from each mating. There were wings resembling club, jaunty, cut, truncate, apterous, beaded, balloon, vestigial, and blistered, and some flies with no wings at all. None of these forms except vestigial could have been due to contamination from other mutant-wing stocks because up to that time none of these other mutants had been brought to the laboratory at the UNIVERSITY OF MICHIGAN.

A definite selection experiment was carried on from March to November, 1920, in an attempt to establish a race of some one definite type of wing character from this mixture, but this attempt was unsuccessful. Flies somatically identical, when bred together, failed to produce offspring that resembled the parents any more closely than did the general population of abnormal flies. Of a total of 6769 flies from matings of like abnormal parents, 5881 normal flies and 1588 abnormal flies were produced.

The curly-winged type of fly appeared among the irregular offspring; and because it seemed a more stable character than any of the others, it was isolated and inbred. At first normal flies appeared in the stock bottles with curly. However, after nearly a year of mass selection for the curly character with an ever-decreasing proportion of the normal-winged type, a 100-percent stock of curly flies was obtained. No normal flies appeared after that until July, 1921. Investigation of the apparently normal individuals which appeared at that time demonstrated the wild type to be only phenotypically wild, but genetically curly. Such flies are found only in very old dry culture bottles.

In the stock cultures of curly there appeared an occasional vestigial-like or cinnabar-like fly. This phenomenon of the occurrence of vestigial and of cinnabar (c_n^2) may, as is shown later, result from a rare crossing over between a lethal factor linked to vestigial in the homologue of the curly chromosome. Cinnabar² also appears on account of crossing over. It was conceivable that vestigial might have been introduced into the stock by contamination, since stocks of vestigial flies were at that time in use in the laboratory. However, this is not likely. The genetic analysis points rather to the occurrence, by mutation, of a new allelomorph of vestigial (v_e^2), more extreme than vestigial and nearly always sterile.

DESCRIPTION OF CURLY

The general size, shape, and venation of the wings of the average curly flies is essentially that of the wild, but the color of the wing membrane is

paler, and the texture is thinner in curly flies. The veins are much less rigid, and finer, and of a lighter color. There is a tendency toward a scallop on the inner margin of the wing at the termination of the fifth longitudinal vein, and there is usually one long fine wrinkle across the wing from near the termination of the second longitudinal vein to the intersection of the fifth longitudinal vein and the margin of the wing. Sometimes there are two shorter crossed wrinkles instead of the single long wrinkle, and not infrequently several short fine wrinkles extending between the third and fourth longitudinal veins (figures 1 and 2).

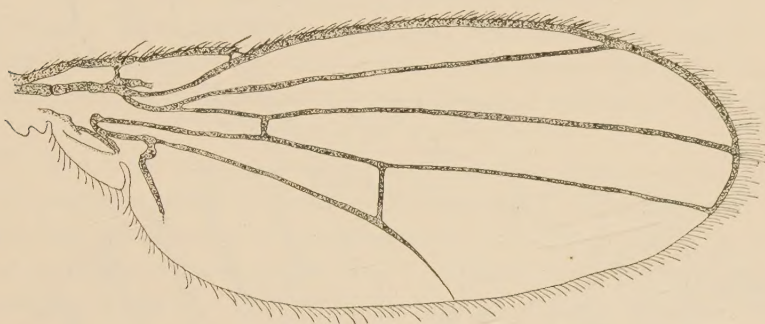


FIGURE 1.—Normal wing of *Drosophila melanogaster*.

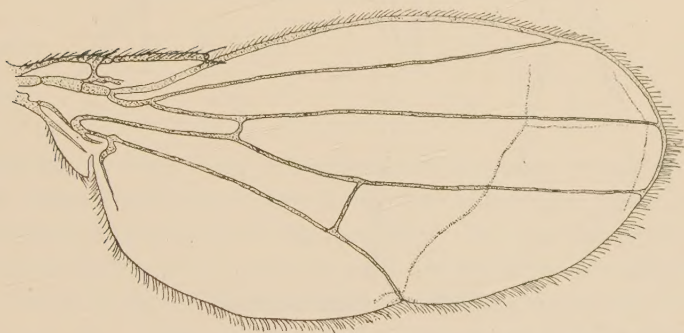


FIGURE 2.—Curly wing, showing cross and longitudinal wrinkles.

The degree of curvature of the wings of curly flies varies from a slight upward bend of the wings at the tip to an extreme roll of 360 degrees or over (figure 3). When the wings are very much curled there are more wrinkles in them, and these extra wrinkles extend both crosswise and lengthwise of the wings.

DOMINANCE AND HETEROZYGOSITY OF CURLY, AND THE PRODUCTION OF
TWIN HYBRIDS

The first test matings using curly were crosses to wild. Either reciprocal cross yielded an F_1 invariably showing a 1 : 1 ratio of curly and



FIGURE 3.—Curly-winged fly.

wild, which is typical of a simple dominant heterozygous character (table 1).

TABLE 1

Offspring from cross of curly to wild.

PARENTS	F_1 CURLY	F_1 WILD	TOTAL
Curly $\times$ wild	1023	991	2014

Additional proof that curly is a heterozygous form was obtained from a mating of the dominant heterozygous star eye with curly. A curly female bred to a star-eyed male gave in F_1 , curly, star, curly star, and wild, just as would be expected were curly a heterozygous dominant character. Further test matings of the curly star F_1 flies together gave an F_2 of curly star flies only. All of these results indicate that curly is a heterozygous form and is lethal when homozygous. BRIDGES informs me that he has raised cultures from F_1 curly stars in which C_y not- S flies occurred in considerable numbers. These C_y flies, which had c_n^2 eye color, were smaller than normal, had especially pinched abdomens, and especially curled wings. When they were out-crossed they were fertile, and all of the offspring were curly of the usual type. This shows that curly can occasionally survive in the homozygous form, and belongs to the class of "semi-lethals."

Curly flies were regarded as simple dominant heterozygotes until a cross was made between vestigial and curly. An F_1 from curly and vestigial gave, not the expected 1 : 1 ratio of curly and wild, typical of the hybrid ratio of a dominant heterozygote and a recessive, but a 1:1 ratio of curly and vestigial (table 2). This result appeared to indicate that the curly fly was heterozygous for vestigial as well as for curly.

TABLE 2

Offspring from the cross of curly and vestigial.

PARENTS	F_1 CURLY	F_1 VESTIGIAL	TOTAL
Curly $\times$ vestigial	512	508	1020

It is later shown that curly is a second-chromosome character, and the suggestion immediately presented itself that a vestigial gene existed in the chromosome homologous to that of curly. To test this hypothesis an F_1 wild male from a cross of curly by wild was mated to a vestigial female from COLUMBIA stock. This F_1 wild male must have contained in it the homologue of the curly chromosome. This cross produced vestigial and wild flies in equal numbers, hence a gene for vestigial must have been carried in the homologue of the curly chromosome.

If the curly stock is heterozygous for vestigial, we might expect to obtain in a culture bottle of curly stock about one-third of the flies with vestigial wings. As a matter of fact the curly stock maintains itself phenotypically pure for curly with no vestigial flies appearing except in rare cases.

These vestigial flies have proved to be sterile in nearly all cases. Of 24 matings (table 3) in which they were used, 6 were fertile and 18 were sterile, and the fertile ones, which were females, hatched only a few (84) offspring.

TABLE 3

Results of the 6 successful matings out of a total of 24, in which only one of the parents in each case was a vestigial segregant.

BOTTLE	PARENTS	OFFSPRING			
		Wild	Vestigial	Cinnabar	Curly
1	$v_g \varphi \times c_n^1 \sigma^1$	3	0	0	0
9	$v_g \varphi \times c_n^1 \sigma^1$	26	0	0	0
13	$v_g \varphi \times C_y \sigma^1$	0	10	0	16
16	$v_g \varphi \times C_y \sigma^1$	0	0	0	2
21	$v_g \varphi \times C_y \sigma^1$	0	0	0	11
24	Real wild $\varphi \times v_g \sigma^1$	16	0	0	0
Totals	84	45	10	0	29

No males were found to be fertile. Professor CLAUSEN reports that he has never found any of these vestigial segregants fertile. This sterility suggests that the mutant is not vestigial itself, but a new and sterile allelomorph (v_g^2).

The failure of the appearance of vestigial flies in the curly stock in large numbers might be explained as the result of the presence of a recessive lethal linked to the gene for vestigial in the homologue of the curly chromosome. Their occasional appearance in the curly stock could be explained by the assumption that they are the result of crossing over between the lethal and vestigial, which thus allows the homozygous vestigial flies to appear. Stock bottles kept at normal room temperature show fewer of these vestigial crossovers than do bottles kept at 30° centigrade in the incubator. As is demonstrated later, high temperature increases the amount of crossing over in the second chromosome, and this fact fits in well with the above hypothesis of the origin of the vestigial flies in the curly stock.

When curly from the balanced stock is crossed to wild, it produces in the F_1 twin hybrids of the types curly and wild. If these types are inbred

separately, the not-curly flies give only not-curlies in each successive generation, but the curly splits up into curly and wild. This parallels the case in *Oenothera*, where *Oenothera Hookeri* $\times$ *Oe. Lamarckiana* produces twins of the types *laeta* and *velutina*, the *laeta* from these crosses splitting in each successive generation into *laeta* and *velutina*, while *velutina* has a constant progeny (DE VRIES 1918).

CHROMOSOME INVOLVED

Since it is established that curly is heterozygous and that vestigial or a vestigial allelomorph is located in its opposite chromosome, and since vestigial is a second-chromosome character, it is evident that curly is in the second-chromosome linkage group. Positive experimental tests to disprove the linkage of curly to first- and third-chromosome genes and to prove the linkage of curly to second-chromosome genes were undertaken.

If the gene for curly were located in the first or X chromosome, then, because curly has been shown to be a dominant heterozygote, a mating between a curly male and a wild female would produce an F_1 of curly daughters and wild sons. That is not the case. In such a cross the sexes are found to be equally distributed between curly and wild flies. The same holds true in a cross of a curly male and an "Xple"² female; that is, a female which is homozygous for the first-chromosome characters, scute, echinus, cut, vermilion, garnet and forked. Here also the curly is found to be present in half of the F_1 males which all show the above six recessive first-chromosome characters. Curly therefore exhibits complete independence of the first or X chromosome (see table 7).

If the gene for curly were located in the third chromosome, it would be found to show linkage with other genes in that group. A back-cross test of curly and the homozygous recessive multiple stock called "IIIple," carrying the third-chromosome recessives, roughoid, hairy, scarlet, peach, spineless, and sooty, was made. As might be expected, the F_1 were one-half curly and one-half wild-type, both types heterozygous for the third-chromosome genes. F_1 curly females were mated to "IIIple" males and the back-cross generation showed complete independence of curly from each of the characters, roughoid, hairy, scarlet, peach, spineless and sooty (see table 8). Because these genes have loci distributed throughout the third chromosome, it is scarcely possible that any linkage with curly would have failed of detection.

² Since it is customary for workers in *Drosophila* to use the terms "Xple," "Iple," and "IIIple" to designate certain mutant stocks, such terms are used here.

An experimental test for the relation of curly to the second-chromosome group involved a septuple back-cross of curly with the homozygous recessive multiple second-chromosome stock called "Iple," which is homozygous for dumpy, black, purple, curved, plexus, and speck. Curly crossed to "Iple" gave an F_1 one-half of which were curly and one-half wild-type, both heterozygous for the "Iple" characters. F_1 curly females were then back-crossed to "Iple" males; and there were produced in the first back-cross generation only curly and "Iple" flies, in the ratio 1:1. This experiment involved many different attempts, and a great number of flies, and always gave the same result.

There was manifested a striking lack of crossing over, for among 2487 back-cross individuals (see table 4), not a single crossover fly appeared. The gene for curly, or some other gene linked to it, modified the crossing over of the "Iple" genes and kept them distinctly associated. The total lack of recombinations of curly with each of these second-chromosome characters showed that curly is a member of the second linkage-group. Crossover flies were finally obtained from curly and "Iple" by subjecting the heterozygotes to a high temperature, as described later in this paper.

BALANCED LETHAL NATURE OF CURLY STOCK

It has been shown that curly is ordinarily lethal when homozygous; likewise, the vestigial allelomorph is linked to a lethal. Since two lethals are present, one in each of the two homologous chromosomes, and since crossing over is practically non-existent in this stock at ordinary temperatures, a condition of "balanced lethals" obtains. This situation is similar to that described by MULLER (1919) in the case of beaded wing in *Drosophila*, and somewhat similar to the situation described by FROST (1920).

Dominant characters that are lethals when homozygous are more easily detected when they occur than recessive lethals. In nature, mutations which are lethal probably arise very frequently and then vanish on account of the process of natural selection. If we suppose, however, that lethals are not left to natural selection, but are arbitrarily selected for by the conditions of an experiment, we may expect them to survive, provided survival is possible.

If some dominant heterozygous character such as curly wing is being selected for, the chances of the survival of any lethal arising in its chromosome or in the one homologous to it are good because of the condition of the "enforced heterozygosity" of the stock. In the selection experiment described above, flies were chosen each generation for the curly wing character and thus kept in such an enforced heterozygotic condition.

Lethals would occasionally arise, and would persist because the stock was kept heterozygous. In fact, if the lethal curly character were selected for and another lethal had arisen in the chromosome homologous to it, that other mutant lethal would be more likely to be saved along with the curly lethal than would the normal gene, because stock containing it would throw a higher percentage of curly flies. In this manner there would be an automatic establishment of a "balancing lethal" for the curly lethal, as there is in selecting for any heterozygous stock in respect to one lethal. If flies containing both of these lethals happen to be taken for parents, a self-perpetuating stock of "balanced lethals" is the result and the stock maintains itself pure for its hybrid constitution.

If the population were only ordinarily hybrid and not balanced lethally, a separation into its respective homozygous strains might be expected to result when the stock is inbred. Flies of the "balanced lethal" type, however, remain necessarily heterozygous for the two lethals and for any other of the genes located in the same chromosome with those lethals unless the balancing effect of the lethals is disturbed by crossing over.

In rare cases mentioned above, in stocks of curly flies which have otherwise continued pure for curly for many generations, it has been noticed that a few vestigial or cinnabar² flies appear. This appearance of the vestigial might be explained if crossing over took place between a gene for vestigial, located in the homologue of the curly chromosome, and a lethal which is present in the same homologue with it. The supposition would be that in the Curly stock, once in several hundred times, crossing over does occur in the chromosome between the gene for vestigial and for a linked lethal. Having thus rid itself of the lethal by crossing over, the vestigial appears. The occurrence of the cinnabar² flies is made possible also by crossing over. It is shown later in this paper that a gene for cinnabar² is present in the chromosome with curly. When crossing over occurs between the gene for cinnabar² and curly, cinnabar² rids itself of one curly gene and can appear in homozygous form in a fly that carries both curly and cinnabar² in one homologue, and only cinnabar² in the other. Such flies, by inbreeding, would produce cinnabar² flies free from curly. Such cinnabar flies, free from curly, have been found and can not be explained as surviving homozygous curly cinnabar.

This phenomenon of the appearance of the vestigial and cinnabar² flies in the curly stock simulates mutation, although it is not really such. In the case of vestigial it is merely the effect of the combination of the two recessive vestigial genes, one of which has lost a linked lethal. In the case of the cinnabar² it is the effect of the combination of the two

recessive cinnabar² genes, one of which has crossed over from curly. The curly cinnabar² flies are in some cases crossovers ($C_y c_n^{+2}$ in one II, c_n^{+2} in the other), and in other cases are surviving homozygotes ($C_y c_n^{+2}$ in both homologues).

The same type of apparent mutation phenomena as the production of vestigial and cinnabar² flies in the "balanced lethal" curly stock had been shown to exist in other "balanced lethal" stocks. MULLER in 1918 demonstrated that the "balanced lethal" beaded stock in *Drosophila* contains recessive factors that sometimes cross over from their linked lethals and become visible because of their homozygosity. On the basis of the beaded results he proposed the same kind of explanation for mutation in *Oenothera*. FROST (1919) found that the same facts hold for *Matthiola*, except that one factor there is not really lethal. G. H. SHULL (1923) states that some of the *Oenothera* mutations also arise by the crossing over of genes linked to lethals which are in a balanced state.

MULLER's theory for the *Oenothera* mutations is supported by evidence from the beaded case, in which the recessive factors which exhibited apparent mutation were artificially inserted. The evidence from curly lends even more support to his theory because in curly the recessive genes arose in the stock in the natural course of selective breeding, indicating the general nature of this phenomenon and its natural occurrence in balanced stocks. Mutation phenomena of the same kind are hence to be expected in nature and must be reckoned with.

CROSSOVER FACTORS IN THE SECOND CHROMOSOME WITH THE GENE FOR CURLY

Since the gene for the production of curly wing is in the second chromosome, the best cross to use for testing the linkage relations of the curly factor to the other factors in the second chromosome is one involving the homozygous recessive multiple second-chromosome stock called "IIple." This stock contains the factors for dumpy wing, located at 9 on the second chromosome, black body at 47, purple eye at 53, curved wing at 78, plexus wing venation at 98, and speck body at 105. These "IIple" genes give checks on six different loci of the second chromosome.

A curly female was first crossed to a "IIple" male, producing in F_1 the classes curly and wild in equal numbers. These F_1 flies were heterozygous for the "IIple" genes. An F_1 curly female was then crossed back to a "IIple" male, producing a back-cross generation which showed unexpected results. Instead of a general reassortment of characters of the "IIple" genes, there were no recombinations at all. What is probably

to be regarded as a 1:1 ratio of curly and "IIple" flies was obtained, which demonstrates very clearly the suppression of crossing over due to curly or to some gene or genes linked to it. Trial after trial of this same sort of mating was made under normal conditions, and there were no crossovers between curly and any of the "IIple" genes (table 4).

TABLE 4

Back-cross to test for the crossing over of curly and the "IIple" genes, using an F_1 curly female heterozygous for "IIple," and a "IIple" male.

DATE	CURLY $\frac{C_y}{\text{"IIple"}}$	"IIple"	CROSSEOVERS	TOTAL
June 1921.....	151	74	0	225
July 1921.....	126	113	0	239
August 1921.....	368	257	0	625
September 1921.....	723	262	0	985
October 1921.....	299	114	0	413
Grand total.....	1667	820	0	2487

The ratio of the total number of "IIple" flies to that of the curly, designated in this table, gives an aberrant proportion on account, undoubtedly, of the low viability of the "IIple" flies in contrast with the extremely high viability of the curly flies. It has been noticed that where curly and wild flies are growing side by side in bottles in the same environment, if adverse conditions arise, the wild flies die before the curly. The "IIple" flies are much more susceptible to harmful external conditions than the wild flies, as is characteristic of mutants, and it is apparent that in a generation in which both curly and "IIple" flies are produced, many of the "IIple" flies might die and the curly would live.

In some of the bottles where conditions were good, as judged from the appearance of the food and the general vigor of the flies, a 1:1 ratio of curly and "IIple" flies was produced. All of these flies were raised in vials, following the method used by CLAUSEN. But CLAUSEN has recently shown, by comparative tests, that, unless these vials are fed with several times as much food as was customary, the ratios obtained are very poor. This is especially true where vestigial is involved, and is also true of "IIple" and "Xple." Unfortunately this defect of the vial method was not known at the time these experiments were made, and so the ratios depart rather widely from expectation in some instances. With this in mind, only conclusions that are unaffected, or are only affected in degree, by the aberrations, have been drawn from the data.

The important feature of this experiment is not the ratio of curly to "Iple" flies, but the fact that in a total of 2487 flies obtained from the curly "Iple" back-cross, not a single crossover occurred. The significance of the fact becomes greater when one considers the possibility of the large number of different combinations such a seven-point back-cross as this one presents. The curly gene, or more probably some gene or genes linked to it, possesses the power to affect the crossing over in the second chromosome.

It is later demonstrated in this paper that there are at least two such "little crossover" factors present in the curly chromosome, one governing crossing over in the left end of the chromosome (table 6) and one governing crossing over in the right end (table 5). Curly crossover flies which were finally obtained under high temperature had lost the "little crossover" factor from the right end of the chromosome, and when mated displayed about the usual amount of crossing over in that region. The curly crossover flies $\left(\frac{C_y \ p_r \ c \ p_x \ s_p}{+ + + + +} \right)$, heterozygous for purple, curved, plexus, and speck, were mated to males of the same genetic constitution. Table 5 gives the number of crossovers obtained. Since crossing over takes place to a considerable extent in the right end of the second chromosome with curly when the original right end is missing, and does not take place under normal temperature conditions when the original right end is present, there is a factor in the right end that prevents crossing over. The factor may be called C_{CR}

TABLE 5

Offspring from two flies each heterozygous for curly, purple, curved, plexus, and speck. Here the curly chromosome contains the left end of the original curly chromosome and the right end of the "Iple" chromosome.

Parents: $\frac{C_y \ p_r \ c \ p_x \ s_p}{+ \ + \ + \ + \ +} \text{♀} \times \frac{C_y \ p_r \ c \ p_x \ s_p}{+ \ + \ + \ + \ +} \text{♂}$

No crossing over.....	{ Curly Wild	32 107
Crossing over between	Visible characters	Number of flies
2, p_r and c	$C_y \ c \ p_x \ s_p$	3
3, c and p_x	$C_y \ p_x \ s_p$	8
4, p_x and s_p	$C_y \ s_p$	4
2, 3	$C_y \ c$	2
3, 4	$C_y \ p_x$	2
1, 2, 4	$C_y \ p_r \ s_p$	4

To test for crossing over between star and curly, F_1 star curly females, having star in one second chromosome and curly in the other, were mated to wild males and put in a temperature of 30°C . No crossovers were obtained from 877 flies (table 6).

TABLE 6

Females heterozygous for star and curly in opposite chromosomes crossed to wild males and kept in a temperature of 30°C exhibit no crossing over.

NON-CROSSEOVERS		CROSSEOVERS	TOTAL
Curly	Star	Curly star	
448	429	0	877

The flies were reared at 30°C for a length of time necessary to affect the germ cells and to partly counteract the effect of the crossover factor C_{CR} in the right end of the curly chromosome. No crossing over occurred under these conditions between star and curly; therefore it appears that there is another crossover factor in the left end of the curly chromosome which is not affected by a temperature of 30°C and which prevents crossing over between star and curly. This crossover factor may be called C_{CL} .

Added proof of the presence of C_{CL} in the left end of the curly chromosome was obtained. Crosses were made in a temperature of 30°C , using curly crossover flies possessing the left end of the curly chromosome but not the right. The effect of the crossover factor C_{CR} in the right end of the curly chromosome was therefore eliminated. Curly purple curved plexus speck crossovers were mated to star flies, producing in F_1 some star curly flies. F_1 star curly females were crossed to wild males, producing classes star and curly but no crossovers, star curly, or wild. Of 518 flies, 264 were curly and 254 star.

EFFECT OF THE CROSSEOVER FACTORS ON CHROMOSOMES I AND III

Since the crossover factors affect crossing over in the second chromosome, it was decided to test their behavior with regard to the first chromosome. It is known that crossover factors in one chromosome may modify the crossing over in other chromosomes. This is illustrated by the action of C_{III-II} , a gene in chromosome III that modifies crossing over in chromosome II (STURTEVANT 1919), and by C_{II-III} , a second-chromosome gene that modifies crossing over in the third chromosome (BRIDGES, unpublished).

Matings were made between curly females and males from the double recessive multiple stock of first-chromosome characters called "Xple." This stock contains the genes for scute bristles, locus at 0, echinus eye at 5.5, cut wing at 20, vermilion eye at 33, garnet eye at 44.5, and forked bristles at 56.5 (BRIDGES 1921). An F_1 curly female heterozygous in her first chromosome for the "Xple" genes, and in her second chromosome for curly and for the "little crossover" factors, was back-crossed to an "Xple" male to test for crossing over.

One hundred ninety-three flies were counted and the crossover classes were present in sufficient numbers to show that the crossover factors in

TABLE 7
Back-cross of F_1 curly females to "Xple" males.

	VISIBLE CHARACTERS	NUMBER OF FLIES
No crossing over	Curly "Xple"	12
	Curly	66
	"Xple"	7
	Wild	64
Crossing over between		
1, s_c and e_c	$C_y s_c$	2
	$C_y e_c c_t v g f$	1
	s_c	1
2, e_c and c_t	$C_y s_c e_c$	2
	$s_c e_c$	2
3, c_t and v	$C_y s_c e_c c_t$	1
	$v g f$	1
4, v and g		0
5, g and f	$C_y f$	3
	f	2
1, 2	e_c	3
1, 4	$C_y s_c g f$	1
1, 5	$C_y s_c f$	1
	$s_c f$	1
2, 3	$C_y c_t$	3
	c_t	3
2, 4	$c_t v$	1
2, 5	$s_c e_c f$	4
3, 4	$s_c e_c c_t g f$	1
3, 5	$s_c e_c c_t f$	1
	$v g$	2
4, 5	g	3
1, 2, 4	$e_c g f$	1
1, 2, 5	$e_c f$	2
2, 3, 4	$c_t g f$	1
2, 3, 5	$C_y c_t f$	1
Totals		193

the second chromosome have no great effect upon crossing over in the first chromosome (table 7).

The small number of flies in the expected classes, curly "Xple" and "Xple," recorded in table 7 is due to low viability of "Xple" flies in contrast to the curly and wild under the conditions of culture.

When a calculation of the percentages of crossing over is made from the data of table 7, it is found that the total amount of crossing over in section 1 is 6.7 percent, as compared with standard 5.5 percent; in section 2, 11.9 *versus* standard 14.5; in section 3, 7.3 *versus* 13.0; section 4, 4.2 *versus* 11.5; section 5, 10.9 *versus* 12.0. Of these values the only one significantly different from standard is 4 (4.2 *versus* 11.5) and probably 3 (7.3 *versus* 13.0). But when the data are looked at more closely, it is seen that this crossing over involves many times as much double and triple crossing over as in the standard condition. This fact, together with the differences in sections 4 and 3, means that the presence of curly and its associated modifiers has had a definite modifying effect upon crossing over in the X chromosome. The amount by which this effect differs from the standard is not so certain because of the small numbers raised and because of the marked disturbances to viability from the use of the vial method.

In a manner similar to that employed in testing the effect of the cross-over genes on the first chromosome, experiments to test their behavior in regard to the third chromosome were performed. Matings were made, using curly females and males from the homozygous recessive multiple stock of third-chromosome characters called "IIIple." This stock contains the genes for roughoid eye, locus at 0, hairy body at 26.5, scarlet eye at 43.8, peach eye at 48.0, spineless bristles at 58.5, and sooty body at 70.7 (MORGAN, STURTEVANT and BRIDGES 1922, page 326).

An F₁ curly female heterozygous for the crossover factors and for the genes of "IIIple," was back-crossed to a "IIIple" male to test for crossing over. One hundred and fifty flies were counted, as is shown in table 8.

The percentages of crossing over calculated from the data of table 8, as compared with the standard, are given in table 9.

From this comparison it is apparent that there has been a very great increase in crossing over in section 3. The observed value is nearly six times the standard, and is the largest instead of the smallest of those involved. Section 1 showed only about half the standard amount of crossing over. The other values are nearly standard. From these data, and from the symmetry of the distribution of crossing-over effects observed by BRIDGES (1920) for the third chromosome, it appears that the crossing-over modifiers of curly increase crossing over in the middle of

TABLE 8

Back-cross of F₁ curly females to "IIIple" males.

	VISIBLE CHARACTERS	NUMBER OF FLIES
No crossing over	<i>Cy</i> "IIIple"	12
	<i>Cy</i>	21
	"IIIple"	12
	Wild	21
Crossing over between		
1, <i>r_u</i> and <i>h</i>	<i>Cy r_u</i>	6
	<i>Cy h st p^p s₈ e⁸</i>	3
	<i>r_u</i>	1
	<i>h st p^p s₈ e⁸</i>	1
2, <i>h</i> and <i>st</i>	<i>Cy st p^p s₈ e⁸</i>	1
	<i>Cy r_u h</i>	3
	<i>st p^p s₈ e⁸</i>	2
	<i>r_u h</i>	2
3, <i>st</i> and <i>p^p</i>	<i>Cy p^p s₈ e⁸</i>	7
	<i>Cy r_u h st</i>	4
	<i>p^p s₈ e⁸</i>	8
	<i>r_u h st</i>	3
4, <i>p^p</i> and <i>s₈</i>	<i>Cy s₈ e⁸</i>	5
	<i>Cy r_u h st p^p</i>	2
	<i>Cy e⁸</i>	2
5, <i>s₈</i> and <i>e⁸</i>	<i>e⁸</i>	1
	<i>r_u h st p^p s₈</i>	1
1, 2,	<i>Cy r_u st p^p s₈ e⁸</i>	2
1, 5,	<i>r_u st p^p s₈ e⁸</i>	1
1, 5,	<i>Cy r_u e⁸</i>	2
2, 3,	<i>Cy r_u h p^p s₈ e⁸</i>	7
2, 3,	<i>r_u h p^p s₈ e⁸</i>	3
2, 4,	<i>st p^p</i>	1
2, 5,	<i>Cy r_u h e⁸</i>	2
3, 4,	<i>r_u h e⁸</i>	1
3, 4,	<i>Cy p^p</i>	1
3, 5,	<i>Cy r_u h st e⁸</i>	2
3, 5,	<i>r_u h st e⁸</i>	1
3, 5,	<i>p^p s₈</i>	1
4, 5,	<i>Cy s₈</i>	1
4, 5,	<i>r_u h st p^p e⁸</i>	1
4, 5,	<i>s₈</i>	1
1, 4, 5,	<i>r_u s₈</i>	1
2, 4, 5,	<i>Cy st p^p e⁸</i>	1
2, 4, 5,	<i>st p^p e⁸</i>	1
3, 4, 5,	<i>Cy p^p e⁸</i>	1
3, 4, 5,	<i>p^p e⁸</i>	1
Total		150

the chromosome very greatly, that this effect falls to normal not far from the middle on each side, and then falls below normal in the ends. In these data there is the same large disproportion of double and of triple crossovers as is the case in chromosomes I and II. These results are also questionable from the small numbers obtained, and from the use of the vial method. Another source of error not adequately controlled was the

TABLE 9

Crossing-over section.....	1	2	3	4	5
Values from table 8.....	11.3	18.0	26.0	11.3	14.0
Standard values.....	26.5	17.0	4.2	10.0	12.5

age of the mothers. BRIDGES finds (unpublished data) that the first output of eggs of females heterozygous for third-chromosome genes shows an abnormally high amount of crossing over in section 3, and that in the offspring of very old females, this may rise as high as the percentage observed here. However, it is believed that the deviations of table 9 do show an effect upon crossing over in chromosome III by the modifiers associated with curly.

INCREASE OF CROSSING OVER DUE TO A HIGHER TEMPERATURE

No crossing over occurred between curly and any of the "I^{iple}" genes in a curly "I^{iple}" back-cross until July, 1921, when the flies were kept in an incubator at a high temperature at Woods Hole. It was not possible to keep the degree of temperature constant on account of lack of equipment, but a temperature of from 25°C to 30°C was attempted. After two generations in the incubator, crossovers were found between black and purple, and purple and curved. Flies from bottles in the incubator were taken as parents for the same back-cross and were carried around in a traveling bag for four weeks. From these bottles no crossover flies appeared. It looked as though temperature were the factor responsible for the crossing over, and an experiment was commenced involving temperature differences.

F₁ curly females heterozygous for the "I^{iple}" characters were again used in a back-cross and were placed in the incubator in a temperature of 26°C. Bottles were likewise placed outside the incubator in ordinary room temperature of about 20°C for a control. No crossovers were produced in any of the bottles either inside or outside of the incubator (table 10).

TABLE 10

Back-cross of F₁ curly females by "IIple" males.

TEMPERATURE	CURLY	"IIple"	CROSSOVERS	TOTAL
20°C	335	242	0	577
26°C	203	25	0	228

A new experiment was started and the temperature of the incubator raised to 30°C. The most favorable degree of heat for normal *Drosophila melanogaster* is said to be about 25°C. In working with curly flies, I find that they thrive in 30°C and show an increase of viability in that temperature. Table 11 gives numbers of flies of various combinations of characters hatched at 30°C, and table 12 shows the percentages of crossing over as calculated from table 11. Even at the high temperature the crossing over is not as frequent as it is in the same regions in other stocks than curly. A total of 1075 flies from this curly × "IIple" back-cross produced 38 crossovers. Of these an abnormally high proportion were double and triple crossovers. The greatest proportional increase in crossing over took place between black and purple, and between plexus and speck. Crossing over between curved and plexus was least increased by the high temperature.

TABLE 11

Back-cross of F₁ curly females by "IIple" males at 30°C.

	VISIBLE CHARACTERS	NUMBER OF FLIES
No crossing over	"IIple"	409
	Curly	628
Crossing over between		
2, b and p _r	C _y p _r c p _x s _p	7
	T ^{dy} b	2
3, p _r and c	C _y c p _x s _p	6
	T ^{dy} b p _r	2
4, c and p _x	C _y p _x s _p	1
5, p _x and s _p	C _y s _p	3
2, 3	C _y p _r	6
2, 5	T ^{dy} b s _p	1
3, 4,	C _y c	2
2, 3, 5,	C _y p _r s _p	1
2, 4, 5,	C _y p _r c s _p	4
3, 4, 5,	C _y c s _p	3
Total		1075

TABLE 12

Percentages of crossing over in each of the regions of the second chromosome tested in the experiment shown in table 11.

CROSSING OVER BETWEEN	TOTAL CROSSOVERS	PERCENT	STANDARD PERCENT
2, <i>b</i> and <i>p_r</i>	21	1.95	6.0
3, <i>p_r</i> and <i>c</i>	20	1.86	21.0
4, <i>c</i> and <i>p_x</i>	10	0.93	25.0
5, <i>p_x</i> and <i>s_p</i>	12	1.11	6.5
Total		5.86	58.5

CROSSOVER FACTORS IN THE HOMOLOGUE OF THE CURLY CHROMOSOME

In an attempt to locate a lethal linked to the gene for vestigial in the homologue of the curly chromosome, the presence of a "little crossover" factor was discovered in that chromosome. From a P_1 mating of a star male and a curly female, F_1 star not-curly females were used for back-crosses to curly males. Only the non-crossover classes, star curly, star, and curly, were present. No wild-type, star vestigial, or vestigial flies appeared. Therefore we may justifiably assume that no crossing over occurred (table 13). Some gene or genes located in the chromosome carrying the gene for vestigial is responsible for the non-crossing over of genes within the second chromosome. From the early history of the curly stock it seems probable that the curly mutation arose in a stock that was already homozygous for these crossing-over modifiers, and that the modifiers present in the vestigial-bearing homologue are the same as those present in the curly homologue. This becomes practically a certainty when the effect of temperature upon the crossing-over modifiers of the not-curly homologue are compared with those of the curly homologue.

TABLE 13

Cross of star females heterozygous for the homologue of the curly chromosome, and curly males, in a temperature of 20°C.

STAR CURLY	STAR	CURLY	TOTAL
132	66	175	373

EFFECTS OF TEMPERATURE ON THE CROSSOVER FACTORS IN THE HOMOLOGUE OF THE CURLY CHROMOSOME

To test for the effect of temperature on the crossover factors in the homologue of the curly chromosome, F_1 star females heterozygous for the

homologue of the curly chromosome were back-crossed to curly males and placed in a temperature of 30°C. All the non-crossover classes, star, star curly, and curly, were produced, and the crossover classes, star vestigial, and wild. Table 14 gives the results of this cross. Of a total of 121 flies, 22 were crossovers besides those star curly and curly flies which are included in the non-crossover classes in the table. Since there was no crossing over under normal temperature conditions, and some crossing over in the high temperature, the high temperature is the factor responsible for the partial counteraction of the effect of the crossover factor in the homologue of the curly chromosome. This behavior is similar to that found for the crossing-over modifiers of the curly homologue and shows that the two sets are most probably the same.

The cross of table 14 shows, by the absence of the vestigial class, that the order of the genes is star, lethal-vestigial, vestigial. Furthermore, since the data of table 12 shows that there is only negligible crossing over to the left of black, the locus of the lethal is about half-way between the loci for black and for vestigial, or at about 10 units to the left of vestigial.

TABLE 14

Cross of star females heterozygous for the homologue of the curly chromosome, and curly males, in a temperature of 30°C.

	VISIBLE CHARACTERS	NUMBER OF FLIES
No crossing over.....	<i>S C_y</i>	41 These include crossovers
	Curly	42
	Star	16
Crossing over between		
1, <i>S</i> and <i>l_{vg}</i>	Wild	10
2, <i>l_{vg}</i> and <i>v_g</i>	<i>S v_g</i>	12
1, 2,	<i>v_g</i>	0
Total		121

LOCUS OF THE GENE FOR CURLY

The 38 crossover flies obtained from the back-cross between curly and "I¹ple" at 30°C (see table 12) showed no crossing over between curly and black. No curly black fly appeared in my experiments, but Professor CLAUSEN reports one curly black and two curly black purple flies in a total of 1325 flies of a curly "I¹ple" back-cross generation. These data

show that curly is to the left of black. If curly were located to the right of black, black curly purple double crossovers could hardly be obtained, for the distance is too short. If it were not for the influence of the "little-crossover" factor in the left end of the curly chromosome, which prevents crossing over of genes in that end, more data might be obtained to locate the exact position of curly in the second chromosome.

CINNABAR², A CINNABAR ALLELOMORPH IN THE CURLY CHROMOSOME

To show that the curly and the vestigial genes of the curly stock were in different homologues, matings were made between F_1 star curly dichaete flies, from a cross of curly by star dichaete, and cinnabar vestigial. Cinnabar, a bright vermilion eye color, is a new second-chromosome recessive located to the right of purple at 55.5. It was found by Professor CLAUSEN, but he has not yet published his results. If curly and vestigial separate at reduction, such a cross of an F_1 star curly dichaete fly and a cinnabar vestigial fly should give an F_2 that contains no vestigial flies. This was the case; no vestigial flies appeared, but an unexpected new feature appeared, in that all the curly flies had cinnabar-like eyes. Cinnabar being a recessive character, this peculiar phenomenon could not have taken place unless the curly chromosome has a deficiency for cinnabar, or carries a gene for cinnabar, or an allelomorph of cinnabar.

The color of the cinnabar eye is very similar to vermilion, and these curly cinnabar-like flies were not quite as bright in color as flies homozygous for cinnabar. On account of this difference in color, the existence of a cinnabar-deficiency in the curly chromosome might have been doubted from the first. However, since the "deficiency" idea fitted in well with the lethal effect of the curly chromosome when homozygous, further evidence was obtained by testing for deficiency.

Cinnabar is located at 55.5, purple at 53, and safranin at 58. If the curly chromosome is deficient for cinnabar, it might be deficient also for purple or safranin or for both. Curly flies were crossed to homozygous purple and to homozygous safranin flies, but in neither case did any of the F_1 curly flies show the eye color of the homozygous parent. No deficiency for purple or safranin, therefore, exists in the curly chromosome.

The dumpy black crossover flies obtained from a curly $\times$ "IIple" back-cross at high temperature, contains the right end of the curly chromosome in one of the second chromosomes:

$$\frac{T^{dy} b \text{ (right end of curly chromosome)}}{T^{dy} b \ p_r \ c \ p_x \ s_p}$$

If no deficiency exists in that chromosome, it must possess a gene for cinnabar, or its allelomorph, in heterozygous form. A simple cross of two such flies will serve to demonstrate whether or not such a gene is really present in the curly chromosome. Crossovers,

$\left(\frac{T^{dy}b \ c_n^{2?}}{\text{"Iple"}} \text{ } \varnothing \times \frac{T^{dy}b \ c_n^{2?}}{\text{"Iple"}} \text{ } \sigma \right)$, mated together, produced 55 "Iple,"

30 dumpy black cinnabar flies, and 40 dumpy black flies. The presence of cinnabar-like flies (homozygous) showed that there is no lethal factor in the right end of the curly chromosome, and since there is no lethal effect there is no deficiency.

The color of these homozygous cinnabar-like flies was not nearly as bright as that of Professor CLAUSEN's cinnabar, and was not as bright as the cinnabar of the curly flies of the back-cross discussed above. This was taken to prove that the cinnabar gene in the curly chromosome is not the same as the original cinnabar but is an allelomorph that produces less effect. The intermediate color of the eyes of the curly cinnabars is due to the fact that they are "compounds" between the bright cinnabar and the dull allelomorph. A further test of the character of this cinnabar allelomorph, cinnabar², was made by mating heterozygous dumpy black cinnabar² flies to cinnabar. One-half of the offspring had cinnabar eyes (c_n - $c_n^{2?}$ compounds), that were not quite as bright as CLAUSEN's cinnabar, and the other half had wild-type eyes. Homozygous dumpy black cinnabar² flies were mated to cinnabar, and all of the offspring had cinnabar eyes not quite as bright as the cinnabar parent.

EVALUATION OF CURLY

Curly is a dominant heterozygous character which is easily recognizable. These facts make it valuable for identification purposes in genetic work on *Drosophila melanogaster*. In normal temperature the "little-cross-over" factors contained in the curly chromosome restrict all crossing over along the entire length of the second chromosome, and keep genes in the two chromosomes from crossing over with each other. When prevention of crossing over is desirable, curly flies may be used. No microscope is necessary in separating curly-winged flies from others, as the upturned wings attract attention at once. Curly flies possess a very high viability and can live in conditions adverse to the health of the wild fly. They are indeed much hardier than any other stocks that I have observed. A pair of curly flies produces a large number of offspring and since practically all flies homozygous for the curly chromosome die, those that do hatch represent a large percent of the number of viable eggs laid. Curly-

winged flies are more easily handled since they are able to fly only in short jumps. They respond quickly to light and are easily transferable from one bottle to another. The genetic and physiological qualities of the curly flies make them especially desirable for experimental work with *Drosophila melanogaster*.

SUMMARY

1. Curly is a definite easily classifiable dominant mutant wing character.

2. The gene for curly is located in the second chromosome. It is usually lethal in homozygous form. The homozygous curly type is easily separable from the ordinary heterozygous type.

3. The homologue of the curly chromosome in the curly stock contains a gene for vestigial and also a lethal. This lethal gives with curly a pure-breeding balanced stock of curly. This balanced stock arose during the course of selection, and is similar in its genetic behavior to beaded, truncate, and double-flowered *Matthiola*. Twin hybrids are produced when curly is crossed to wild stock.

4. Two "little-crossover" factors, one in the left end of the curly chromosome, C_{CL} , and one in the right end, C_{CR} , prevent crossing over in the second chromosome under normal temperature conditions. The effect of the crossover factor in the right end is reduced when the temperature is raised to 30°C, but the one in the left end is not affected by a rise in temperature.

5. The crossover factors in the curly chromosome probably affect crossing over in the first chromosome, and also in the third chromosome.

6. In the vestigial-bearing homologue crossover factors exist that are probably the same as those in the curly homologue.

7. At 30°C curly shows crossing over with black only very rarely, more frequently with purple and with curved. The crossover relations indicate its locus to be to the left of black.

8. The curly chromosome contains a gene for an eye color similar to vermilion but less bright, and allelomorphic to cinnabar; it is designated cinnabar² (c_n^2).

9. The curly second chromosome is valuable genetically on account of the dominance of its curly factor, on account of its low-crossover factors, and its recessive lethal effects.

10. Vestigial and cinnabar² flies occasionally appear in the pure curly stock, simulating mutation, although they are really due to crossing over of vestigial and cinnabar from the balancing lethals.

11. The genetic constitution of all flies in the pure-breeding curly stock may be represented as $\frac{C_{CL} C_y + c_n^2 + C_{CR}}{C_{CL} + l_{vg} + vg^2 C_{CR}}$.

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